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Liew, Choong-Chin

<120> Method for the Detection of Gene Transcripts in Blood and Uses  
Thereof

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<150> US

<151> 2000-01-04

<150> US 60/115,125

<151> 1999-01-06

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<170> PatentIn version 3.2

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25 actcacgac caaactatga attttagatt cggatcaaac gaggattgat ccgagggacc 180  
aacgttga taaatcttac gtcgtcttat atattaagtt tttgtggagg atcggataag 240  
30 tctatagtgt ttgtcacaga tagtcccga ccacacccca gaccatagga gtcgtctcc 300  
ggaccgcggt ctaatggg 318  
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45 caaataaaca aaaacttcca atctccattt aaaat 155

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 10 aaacaccttt ttggtgtcaa gaagaaaaag actagcttca ctgaacagag aaatgctgga 120  
  
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25 gaaaaanttt tccggggggc ccaccggga agggntnccg gggaaggg 168

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30 <212> DNA  
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 cccacattc cattttaat atatactgtg cttacaaat attataatat gttttaaatt 180

40 atgttcacag aagcacctgg tctgtgaatg gcatgccagc attaaaaaa ataagcattc 240  
 tttgaatata tatttagttt ttaattgtg taggaaaatc aaagccagag ggagtagaaa 300

caaaatttgt gattttctaa atactcttg gctgcaggga agaaaccag tcccaggcga 360

45 agtctacct aattgatga taaaattaca tggaagggat tctgttggc atgaggacct 420

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438

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 15 aaagggggaa ggcccccaa accggggggg gggnaaaaag gtgggtttcc cccttttcc 180  
 aattccaaa accaatttc aaaaggnaaa ccaacnttc caaaatggg aaagg 235

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 45 caaattgggg ggnaaaccaa atttaagggg ggaagggggg gncccccccg ggaaaggccc 180



aaggggggaa aatttttccg ggggtgggtn gggggaacca atttaagggg ggggccccg 240

gggggggtcc ccttgggcn ttttcttt tgggtnaaaa aaaaaaaccc ctg 294

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15

ctgtacttga actctaaaac tgttgagaa actcagtgt tacccaaca gattcatttc 180

aaatagctgt aaaaggtatg ttactccag aagaccagag ttgcttctt tgaacttctc 240

20

attccttggg cctaggaacc ctcatcacc tcacccaac gtcaaccag atcttctctt 300

ccataaacag cactccctca ggcccctgcc tgacacaggc atagactgtc atgttgatt 360

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tttaggcggc accccaacaa caccaggccc tacttttcc aaggncgggg aagcccatgg 180

45 gttctgggna acgggcaatg cgggcttgca acgggnggaa naaaaacagn cccaaaagaa 240

tg

242

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 tctcagctca ctgcaacctc cacctctcag gagaattgct gaacctggga ggcggaggtt 120  
 15 gcaggagct gagattgcgc cactgccctc catcctgggc gacagagcaa gaacctgtct 180

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181

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 15 aaggggggtg gggaagagcc aaatttctt gggaanaac gcccccttgg ggaaaanaag 180  
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 30 ggaagaattg tatgtgggag ttttaatgg tctcatttca ttggctataa ctcaattaca 180  
 aggagaaata taactgcaga ggagcttga aaatttagt cagctgaggg taaaggaaga 240  
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 40 ttgaggcgaa aggactcgcg gatttgacgc ttgatgcggt tgcgctcgac ggcgagcttg 180  
 acgctctttt tgccgatcac caaacctagg cggggatgat caagctgggt atcgcgcgct 240  
 agcagcagga cacttttgcc cgggagcttt accgcttggg gagtcgaaga ctgccttgna 300  
 45 ttgccgggga gtcagcagtc gctttttccc ggnegaagcc tcgaactcac cancctgtct 360

ggattaatta gacagcaaga cgcttgcggc ccctttggcg cgaacgaacn ncgaaaagga 420  
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 45 gagaaccccg cacaccgaaa ggctaaggat tcctccgcta tgtcaatcaa cggagggtta 180

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 5 tgggcgccag caggggcggc ctcggggcca aaccgaacct caggtcanac ttccaagaaa 360  
 agtgggtgaa acgccagcgc atggcaaccc gtaccgcaaa ccgacacagg tagccggggg 420  
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aagaaggaac ccacctngg ggccaacaa aaacttaaaa acccccccat ttentncccc 180  
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30  
gggtgctcctg cgtttggact gtgcaggcct ctctatgct gatgcgtctc ccactcctg 180  
  
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35 cctgtccctg ctgttcttgg tgcacccag caccaggcca ctctgggagc cagggcagat 300  
  
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tttaaaaatt tatgcatat gtccagcttc cagtcagtgc ttctggtag catgaggata 120

5 actagatttt actgtagatg gtagataaaa gtccagtga aagcaaagat gtgtaatgtt 180

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ccngnctatc tcaactganga tgattgncag ngccnntggg tggcacnang ggnactgatg 360

25 ancancactg accctgccga cgccagangc cgcanatccg gagantncat gngacnatat 420

aggttaccnc cttnaccgg gcancaatct gcttctatgg tgaatgcaga ccatntagaa 480

30 ntctntcnct ataggcatga ttttnnncag tgcgtcagcc tganaanga ancnnacttt 540

tgntagatga nnnngtctc ncccttgngg ctnacaaatt ccancacctt tgggtggcngc 600

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 10 gatgacataa aatgagatga aatgagatgt aatgatggaa tgagatgaga tgaaatgaga 120  
 tgaaatgata gatgagataa aatgatgata tgaaatgatg agatgaatga tgagatgatg 180  
 15 agatgaatga tgaaatgaaa tgatgagatg agatgatgaa atgaaatggt gagatgaaat 240  
 gatgagatga aatgaaatag tgaaatgaaa ttgaaataaa atcgaaatga gagatgaaat 300  
 gatgagatga tgaaattgat gaaatgatga gatgtgatga gatgaaatga tgagatgaga 360  
 20 tgagatgaca tgaaataatg aaatgaaatt gaaatgagat aagatacgag ctgagatgca 420  
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 cacag 125  
  
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 35 aatgaatct gagatgaaat gagatgaaaa ntgatacgaa aatgatata aaaaatatga 180  
 cctgagatga aatgagatga aaaatgatac gaaaaatgat ataaaaata tgacatgaaa 240  
 40 tgaaatgaga tgatatgaaa tgacataatg aatgatgaa ttgatgatat tgaaatgaaa 300  
 ttgaaagatg agatgaaatg atgagatgaa atgaaatgtt gaaatgatga agagatgtga 360  
 catgaaatga gctgaaatga gatgaaatga aatgagatta aatgatgaga tgaaaaatga 420  
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 gaaatgagat gaaataaaat aatgaaatga tgaaataatg aaatgaaaat gagatggaaa 180  
 30 tgatgagatg agaagaaatg atgagatgaa atgatgaaat gatgagatga ganaaaatga 240  
 gatgaaatga tgagatgaga tgaaatatga tgagttgaaa tgacataatg aatgaaatga 300  
 35 tgaaatggaa taatgaaatg gaaatgatga gctgagatgc aatgagttga aatgagatga 360  
 aatgatgaaa tgatgagatg aaatgatgaa atgaaataat gaaatgagat gaaataaaat 420  
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cataaaatga gatgaaatga gatgtaatga tggaatgaga tgagatgaaa tgagatgaaa 180  
20 tgatagatga gataaaatga tgatatgaaa tgatgagatg aatgatgaga tgatgagatg 240  
aatgatgaaa tgaaatgatg agatgagatg atgaaatgaa atggtgagat gaaatgatg 299

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cgccatgaaa ttctgctcg attagcttac gttgtttgga tagaggccaa acaaggctgt 180  
45 tatcggtagc aggaatggat gttcgatttc gtagaatacg cctgagagac ggccaatact 240

ctcacgagag gcagcaggcg cgtaaattac ccaattacaa caagtagagg tagcgaagga 300

aaatatgagg ggtggcaagg tttgcctgt tacattctca aatggaagca aattagatat 360

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gtcattg 367

<210> 77

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<213> Human

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30 tcagtgtttg gggtttattt taaaagaat aggggtgccac cagatgttct ttagtggagg 180

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 nntaantaaa aagggtgaag aannttactt cncctggtec taaaaacnt ttcttcagt 120  
 30 tattacaaa atattggac cattantaaa gantagggcc aaccnaatt ttcttgaaa 180  
 ttccgttaa atagccgta aatgtttta cccatttcat attggatacc ttaaattata 240  
 35 ataatggatt ttattgttaa attgtgtgtg tgtggtgtgt atgccctgtc ttctctctc 300  
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 35 ccttgatggt caagtattta agaccctatg cnaatatttt ttaccttttc taataaacca 120  
  
 tgtttggt 128  
  
 40 <210> 80  
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 ggaaaanaaa tccttttaa anggaantcc aagggaagga ncctgnncaa nacttcccn 180  
 30 ccaataaaaa aaacntttt ggaaangggg aaa 213  
  
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 35 <211> 443  
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<223> n is a, c, g, or t

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tgatgaaatg agatgagatg agaagaaatg acttgatgag atgagataaa atgatgaaat 120

gaaatgaagt gaaatgaaat tgaaatgaga tgagatgaaa tgagataaaa tgatgagatg 180

15 aaatgagaag aaatgagatg aaatgatgaa atgatgagat gagatgaaaa atgatgggat 240

gagaaatgag atgaaatgat gggatgaaat gaaatgaaat aatgaaataa tgaaatgaaa 300

20 tgaattgata atattgaagt gaaattgaaa gatgagattg gatgaaatga tgagatgaaa 360

tgaaatgttg aaatgaaatg aagagatgta acatgaaatg agctgaaatg atgagatgaa 420

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 25 gacctctgaa tgtattantg anncaagtga tagccaagat attttgaaga aaaatagata 120  
 ntagggacct gctctataag cccatcataa ttattatga agttataaca agtaaaacag 180  
 taaggtattt ggcatggaat agagaacca gaaacagacc caatgcatgg gtacaggata 240  
 30 taacacaggg aaatgaggga caatatatgg ttctgggata attattata tggggaaaat 300  
 aaagaaattg gatccctacc tcacacatac aaaaaaaatc ataattgaat taaaaacttg 360  
 35 catgtgaaag gaaagacttt aaaacattta gaaaaagtat tggaggctat gatcttgggg 420  
 taggaaagca ttctttttt tt 442  
  
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15 aggaaggaag gaagg 135

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20 <212> DNA  
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gaggatcctt ctgtaagtgc ccctactatc aagggttcat ggaaatcta ggcaaggcag 120  
  
aacttcctca gaaggacaag agacaaagaa gtgggggagg cctcctatc catagctgag 180  
35 aggggttatt cttgtggtt ctgctgacag agcctttgga tgtctgatct gagatggagc 240  
  
aaccacagct agacagaact tttagattt tgggggggtt aaaaggcctc aagcaaattc 300  
  
40 taaaactttc ttgaacccc ctggcatagg ctgagttcc ctgact 346

<210> 85  
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45 <212> DNA  
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<400> 85

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5 gaaaagaaaa tggtttttt tgccgaaatc aaccgggtaa 100

<210> 86

<211> 201

10 <212> DNA

<213> Human

<400> 86

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15 gaaaagagga agaaacgctt ttaggaggt aaggattaaa gtaaaaatta tttgacata 120

attacctt tttgtacca ctcttaaagg ccaggaacat attggagaa gcctagtgt 180

20 atgtaacagt gtggggtttc a 201

<210> 87

<211> 531

25 <212> DNA

<213> Human

<400> 87

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30 aaccagaac gaacgcgtta agtgcgcgtt atcttctag gatagagtcg gtgacgggaa 120

tcttttacc cggcactcgg gtccaccctc gcggcaccag aggtattctc cggcgagtcg 180

35 ttaaccatcg caatgccga ccgagtttaa ggaccactcc ccaccttct cattagtaa 240

ggagaacgt actttacccc atagacggag aaatcgctac tcaactacca ggcgcgcgcc 300

gtcgagtccc tcttctctc ttatgcatt tagagcgctt tcgtaagagt tttccctaga 360

40 ttttctaag cgtagcgcgt ctactccaat gtttcgtta atccagccc aactaacgcc 420

gcggaggagt cgtccgtct actcctatcc cgtcggctcg gatttactac aggagctaag 480

45 aaaacaaaa gtaccagccc taaaggaaag tcaaaggacg cccgtaaaaa a 531

<210> 88  
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 5 <213> Human  
  
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 acagccaaac cgggtccgcc aaggcctccg tcgtttata atatattccg ttacgtata 180  
 aggaacgaac ccccttcat taccacgggc ccgcgtccgc ctcttctcc attcgcaaca 240  
 15 gttctattcc ttacagctc ccgtacctgc ttccagaaca tcgcaccgcc atagtcgaaa 300  
 gatagcaaag attaccagc ttctattcct cgtcccagag ccgagtaaat cgaagttat 360  
 20 agaggcggaa tccaaccatt caagagttat aacaagttat cggcactcgg gggatcagaa 420  
 tataaactta atgtccctt tatttcccg gacgccctt ttaaccatt ctctctatct 480  
 ttgctaaca agccattgac ggcgctttgc cgcgcgggcc catctcgcgt 530  
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<210> 89  
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 30 <213> Human

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10 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

15 <221> misc\_feature  
<222> (782)..(782)  
<223> n is a, c, g, or t

<220>

20 <221> misc\_feature  
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<223> n is a, c, g, or t

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30 <221> misc\_feature  
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<223> n is a, c, g, or t

<400> 96

35 gagatgaatg atgaaatgat gagatgagat gatgaaatga aatggtgaga tgaactgatg 60

aaatgaaatg aaataatgaa atgaaattga aataaaattg aaatgagatg agatgaaatg 120

atgagatgat gaaataaaat gatgaaatga gatgtgatga gatgaaatga ngagatgaaa 180

40 tgatgagatg agatgacatg aaataaatga aataatgaaa tcgaaatgag atgagaagat 240

acgagatgag atgaaatgat gagatgaaat gatgaaatga gataagatga aaagagttga 300

45 tgagatgatg agatgaaatg agatgaaaag agatgaaatg agatgaaatg aaatgatgag 360

atgaaatgag gtgaaatgaa attagatgaa acgtaatgag atgaaatgac ataataaat 420  
gaaaaaatga aatgaaataa tgaaatgagg tgaaattaaa tgagatgatg aaattaaatg 480  
5 atgaaatgaa ataataaat ggaaatgaaa tggaaatgat gagatgaatg atgagatgaa 540  
atgatgagat gagatgtatt gatgagagga aatgatgaga tgtaatgaaa tgagatgaaa 600  
tgaaatgagat gaaatggaat antggaangg aaattgattg gngatttgag atgaaatgag 660  
10 ntaaatgnga tgaattaatg atgagatgaa atgntgaatg ccgggggtggn tgagatgaat 720  
tgagttgaac cctgngatga atgaagattg nntgaatggt ggntgaatgt tgaatggntg 780  
15 gntggnanaa tgcctgtngg 800

<210> 97  
<211> 334  
20 <212> DNA  
<213> Human

<400> 97  
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25 atgaaatgat attgaaatga aattgaaaga tgagatgatg agatgaaatg gtgaaatgtt 120  
gaaatgaaat gatgaaatga atagatgtga catgaaatga gctgaaatga tgagatcaaa 180  
30 tgaaatgaaa tgagattaaa tgatgagatg aaaactgatg aaaacttaaa tgatgaaata 240  
atgaaatgaa aatgaaatgg aaatgatgag atgagaagaa atgatgagat gagatgagat 300  
aaaatgagat gaaatgatga gatgaaatga tgag 334  
35

<210> 98  
<211> 100  
<212> DNA  
40 <213> Human

<220>  
<221> misc\_feature  
45 <222> (17)..(17)  
<223> n is a, c, g, or t

<400> 98

ttcaggccgt ctgctntac atatactatc gagaatggtg ctgtgcactc ataaccgt 60

5 tgcttggtag acgcttttga acccttcagc gctgaaagta 100

<210> 99

<211> 500

10 <212> DNA

<213> Human

<220>

15 <221> misc\_feature

<222> (8)..(8)

<223> n is a, c, g, or t

<220>

20 <221> misc\_feature

<222> (361)..(361)

<223> n is a, c, g, or t

<400> 99

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agatgaaaag agttgatgag atgatgagat gaaatgagat gaaaagagat gaaatgagat 120

gaaatgaaat gatgagatga aatgaggatga aatgaaatta gatgaaacgt aatgagatga 180

30 aatgacctaa tgaaatgaaa aatgaaatg aaataatgaa atgaggatgaa attaatgag 240

atgatgaaat taaatgatga aatgaaataa tgaaatggaa atgaaatgga aatgatgaga 300

35 tgaatgatga gatgaaatga tgagatgaga tctaataatg agaggagatg atgagatgaa 360

ntgagatgaa aagagatgaa atgagatgaa accgaaatga tgagatgaaa tgaggatgaaa 420

tgaaattaga tgaaacgtaa tgagatgaaa tgacataatg aatgaaaaa atgaaatgaa 480

40 ataataatgaaat gaggtgaaat 500

<210> 100

45 <211> 397

<212> DNA

<213> Human

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<220>

10 <221> misc\_feature  
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<223> n is a, c, g, or t

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15 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

20 <221> misc\_feature  
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<223> n is a, c, g, or t

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25 <221> misc\_feature  
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<223> n is a, c, g, or t

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30 <221> misc\_feature  
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<223> n is a, c, g, or t

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35 <221> misc\_feature  
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<223> n is a, c, g, or t

<220>

40 <221> misc\_feature  
<222> (177)..(177)  
<223> n is a, c, g, or t

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tccccctgaa nntctctaaa ccttaacttt ctggccnttt tgtttcagan atgcctaacc 120  
 ctacagggggt cttttgttct ctacgcctaa aaacttaatc tgtttggaac aattccnttt 180  
 5 cctctctgta gaaattgacc tggccatggc tcctgtgaat gatacgggtg ctattatccc 240  
 tgaacactgt aaaaatgaac ttgaaacag ttgggttaga cccaaacaga aaatgatgta 300  
 tggcttgga atagtttagc tgaacattat gctttaatat ttactggcc attgcagcac 360  
 10 aggtttagaa atttatgttc ggcttttta agtttta 397

<210> 101  
 15 <211> 132  
 <212> DNA  
 <213> Human

20 <220>  
 <221> misc\_feature  
 <222> (121)..(121)  
 <223> n is a, c, g, or t

25 <400> 101  
 gttaccta atgtttactct cttttcttt ttctttattt ttcatgtga aaataggaac 60  
 attaatgta ctactttcaa aagaattaat tgaagaaaga gagatacagg gtatctaggc 120  
 30 ngaggaagac cc 132

<210> 102  
 <211> 246  
 35 <212> DNA  
 <213> Human

<400> 102  
 gggggcttta gttataactg ggctaagcat aattgcgcta ccaattccat attatctcat 60  
 40 ggcactta atttataattg atatatataa taaaaaatc aatgcagata ttgatataat 120  
 aaaaatagat aatggtaatc caagcacgat ggtagccatc actctaattg ctttgggggt 180  
 45 aacctataac ttattaagta aagtccaga atggttctt gacagtatta aaattaaaga 240



aaacag

246

<210> 103

5 <211> 18

<212> DNA

<213> Artificial Sequence

<220>

10 <223> forward primer of exon 1 of insulin gene used for quantitative RT-PCR analysis

<220>

15 <221> primer\_bind

<222> (1)..(18)

<400> 103

gccctctggg gacctgac

18

20

<210> 104

<211> 18

<212> DNA

25 <213> Artificial Sequence

<220>

<223> reverse primer of exons 1 and 2 of insulin gene used for quantitative RT-PCR analysis

30

<220>

<221> primer\_bind

<222> (1)..(18)

35

<400> 104

cccacctgca ggtcctct

18

40 <210> 105

<211> 24

<212> DNA

<213> Artificial Sequence

45 <220>

<223> forward primer of  $\beta$ MyHC gene used for quantitative RT-PCR

analysis

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 <400> 105  
 gctggaacgt agagactccc tgct 24  
 10  
  
 <210> 106  
 <211> 24  
 <212> DNA  
 15 <213> Artificial Sequence  
  
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 <223> reverse primer of  $\beta$ MyHC gene used for quantitative RT-PCR  
 analysis  
 20  
  
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 <221> primer\_bind  
 <222> (1)..(24)  
 25  
 <400> 106  
 ggatccttcc agatcatcca cttg 24  
  
 30 <210> 107  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence  
  
 35 <220>  
 <223> forward primer of ANF used for quantitative RT-PCR analysis  
  
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 40 <221> primer\_bind  
 <222> (1)..(20)  
  
 <400> 107  
 ggatttcaag aatttgctgg 20  
 45

<210> 108  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence

5

<220>  
 <223> reverse primer of ANF used for quantitative RT-PCR analysis

10 <220>  
 <221> primer\_bind  
 <222> (1)..(20)

<400> 108  
 15 gcagatcgat cagaggagtc 20

<210> 109  
 <211> 20  
 20 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> forward primer of APP used for quantitative RT-PCR analysis  
 25

<220>  
 <221> primer\_bind  
 <222> (1)..(20)  
 30  
 <400> 109  
 ggatgcttca tgtgaacgtg 20

35 <210> 110  
 <211> 19  
 <212> DNA  
 <213> Artificial Sequence

40 <220>  
 <223> reverse primer of APP used for quantitative RT-PCR analysis

<220>  
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 <222> (1)..(19)

<400> 110  
tcattcacac cagcacatg

19

5

<210> 111  
<211> 21  
<212> DNA  
<213> Artificial Sequence

10

<220>  
<223> forward primer of ZFP used for quantitative RT-PCR analysis

15

<220>  
<221> primer\_bind  
<222> (1)..(21)

20

<400> 111  
cacargagrc arggtcaacg a

21

25

<210> 112  
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<212> DNA  
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30

<220>  
<223> reverse primer of ZFP used for quantitative RT-PCR analysis

35

<220>  
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<222> (1)..(22)  
<400> 112  
ggattaaaat gaagcaccca ga

22